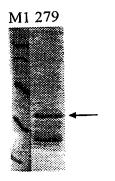
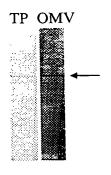
279 (10.5 kDa)

Fig. 2

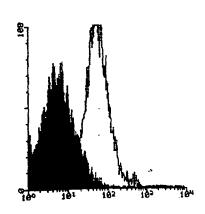
## A) PURIFICATION



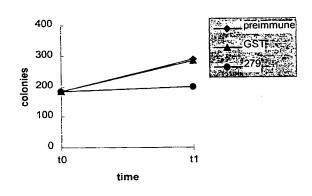
#### **B)WESTERN BLOT**



### C) FACS



### D) BACTERICIDAL ASSAY



E) ELISA assay: positive

#### 279

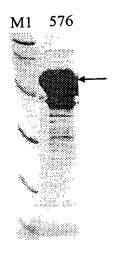
The predicted gene 279 was cloned in pGex vector and expressed in E. coli. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that protein 279 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vescicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

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576 (27.8 kDa)

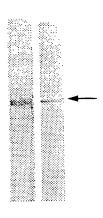
## Fig. 3

## A) PURIFICATION

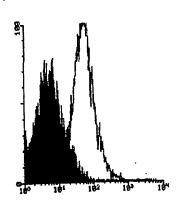


#### B) WESTERN BLOT

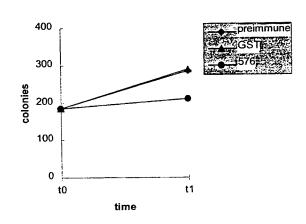
TP OMV



#### C) FACS



## D) BACTERICIDAL ASSAY



#### E) ELISA assay: positive

#### 576

The predicted gene 576 was cloned in pGex vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 576-GST fusion protein purification. Mice were immunized with the purified 576-GST and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 576 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vescicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

519 (33 kDa)

#### A) PURIFICATION

M1 519

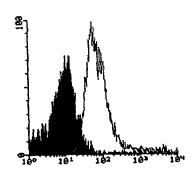
Fig. 4

#### **B) WESTERN BLOT**

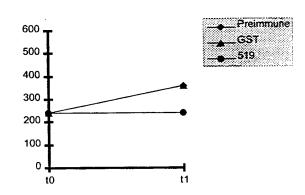
TP OMV

as the professional for the second se

#### C) FACS



### D) BACTERICIDAL ASSAY



E) ELISA assay: positive

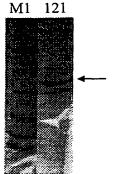
#### 519

The predicted gene 519 was cloned in pET vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 519 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

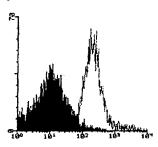
09/674545 PCT/US99/09346

## 121 (40 kDa)

## A) PURIFICATION

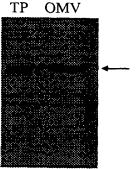


#### C) FACS

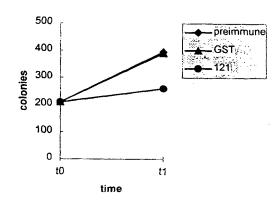


E) ELISA assay: positive

## B) WESTERN BLOT



#### D) BACTERICIDAL ASSAY



#### 121

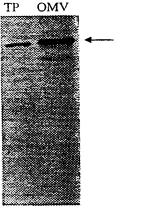
The predicted gene 121 was cloned in pET vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vescicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

## 128 (101 kDa)

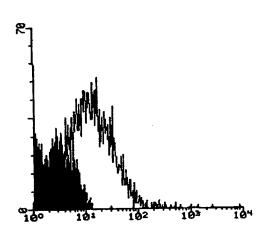
Fig. 6

A) PURIFICATION
M1 128

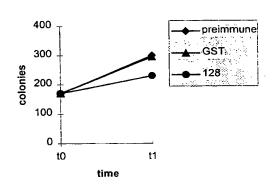
B) WESTERN BLOT



C) FACS



D) BACTERICIDAL ASSAY



E) ELISA assay: positive

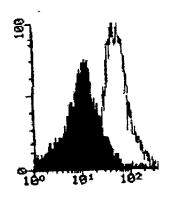
#### 128

The predicted gene 128 was cloned in pET vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

A) PURIFICATION

M1 206

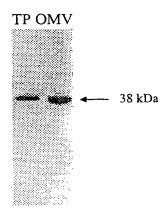
C) FACS



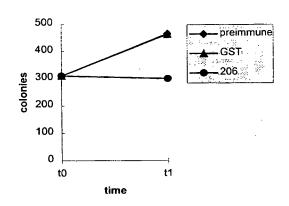
E) ELISA assay: positive

## Fig. 7

## B) WESTERN BLOT



#### D) BACTERICIDAL ASSAY



#### 206

The predicted gene 206 was cloned in pET vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worthnoting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in E. coli without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from E. coli expressing this native form of the 206 protein showed a recative band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

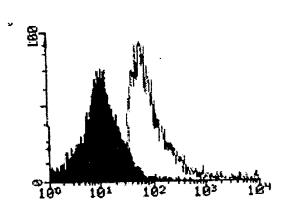
287 (78 kDa)

Fig. 8

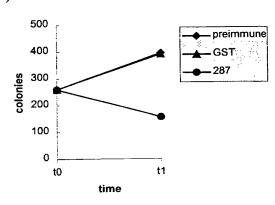
## A) PURIFICATION



#### B) FACS



## C) BACTERICIDAL ASSAY



D) ELISA assay: positive

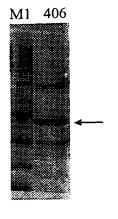
#### 287

The predicted gene 287 was cloned in pGex vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A).

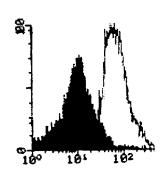
406 (33 kDa)

Fig. 9

A) PURIFICATION

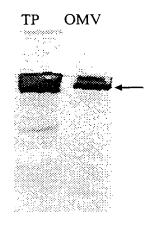


C) FACS

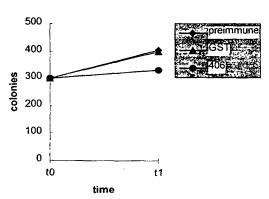


E) ELISA assay: positive

#### B) WESTERN BLOT



#### D) BACTERICIDAL ASSAY



#### 406

The predicted gene 406 was cloned in pET vector and expressed in E. coli. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, N. meningitidis total protein extract; OMV, N. meningitidis outer membrane vescicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the N. meningitidis immunoreactive band (B).

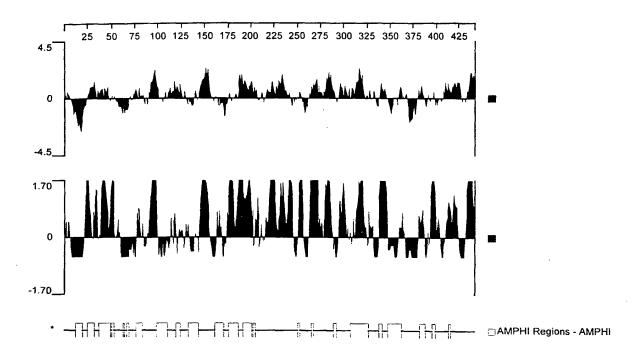


Fig. 10

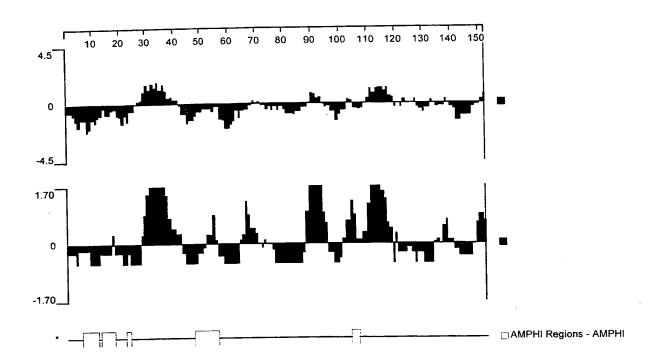


Fig. 11

11/30 <u>576-1</u> Hydrophilicity Plot, Antigenic Index and AMPHI Regions

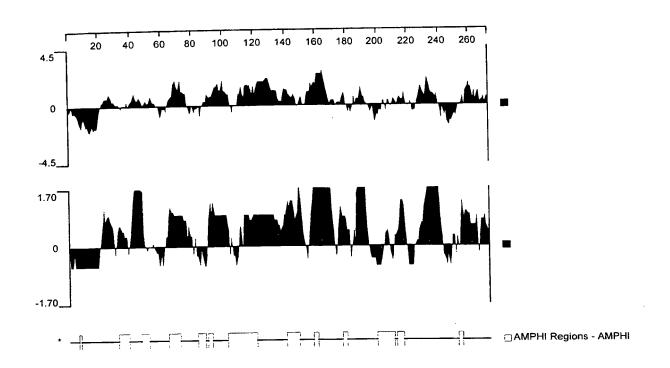


Fig. 12

12/30 <u>519-1</u> Hydrophilicity Plot, Antigenic Index and AMPHI Regions

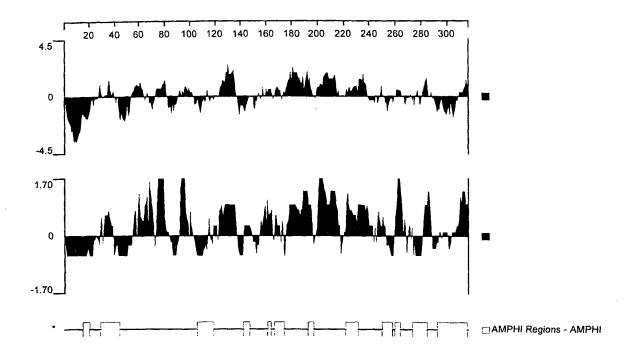


Fig. 13

13/30 121-1

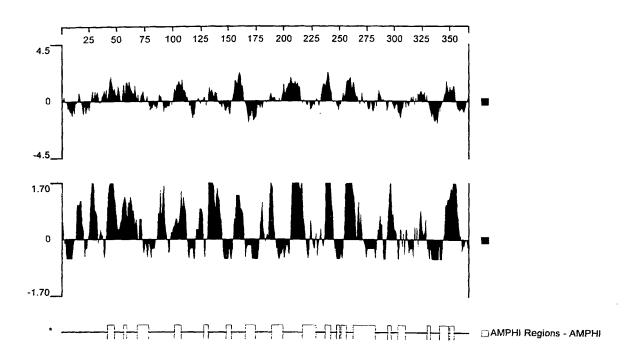


Fig. 14

WO 99/57280

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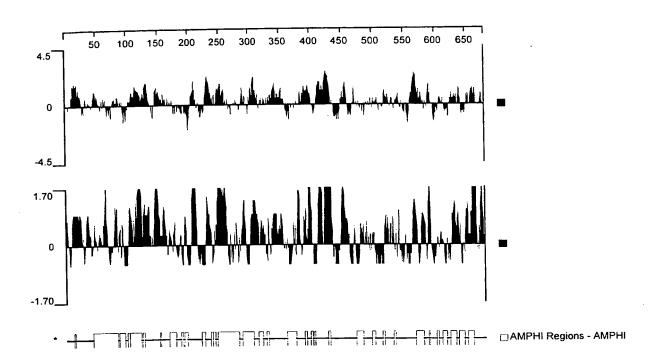


Fig. 15

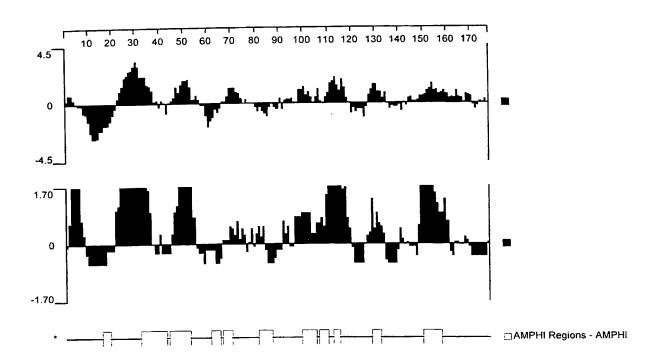


Fig. 16

16/30 **287** 

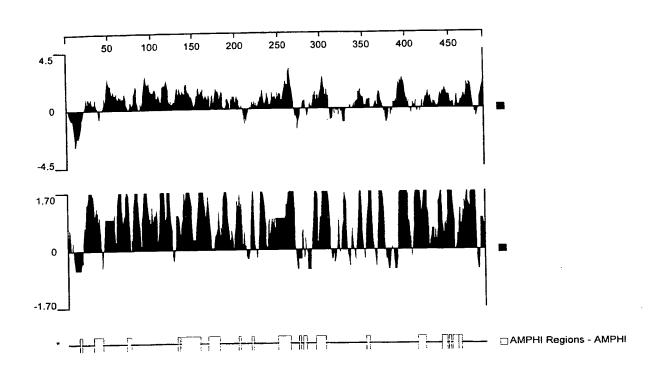


Fig. 17

WO 99/57280

17/30 **406** 

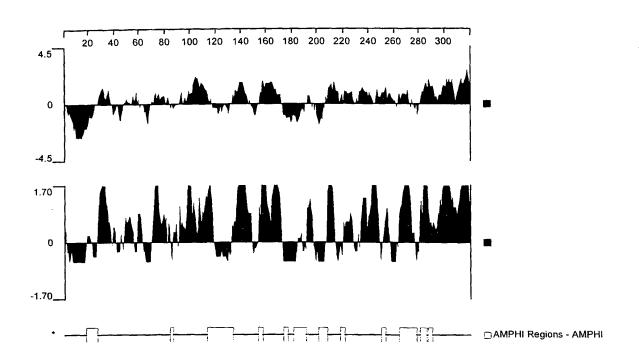


Fig. 18

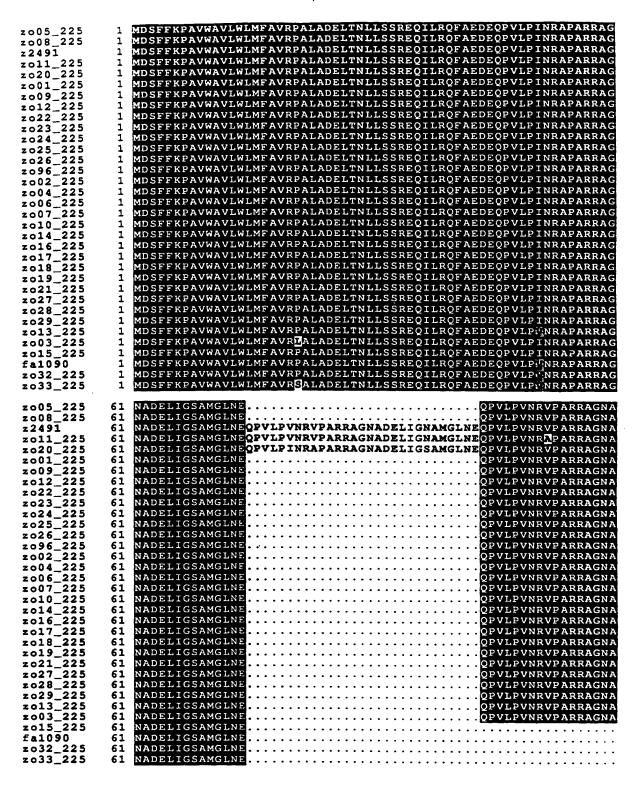


Fig. 19A

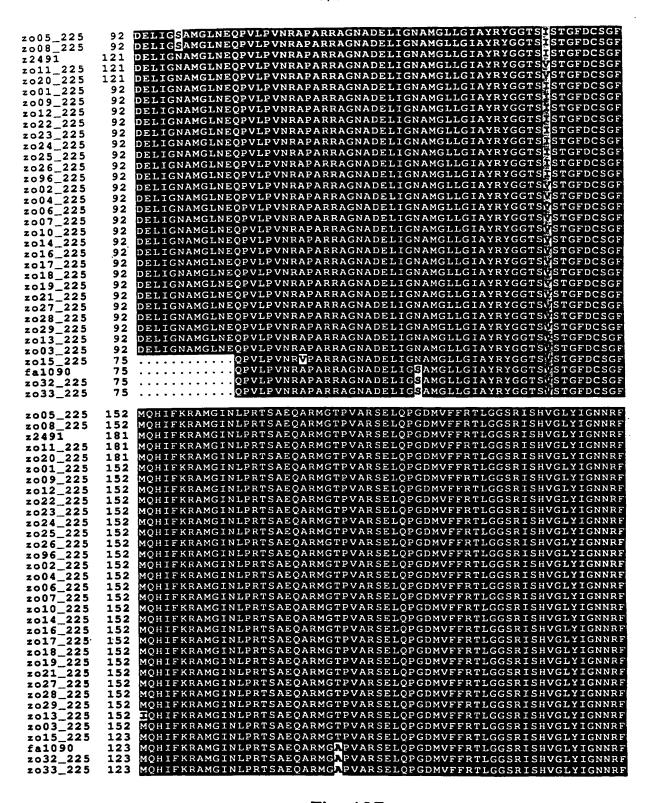


Fig. 19B

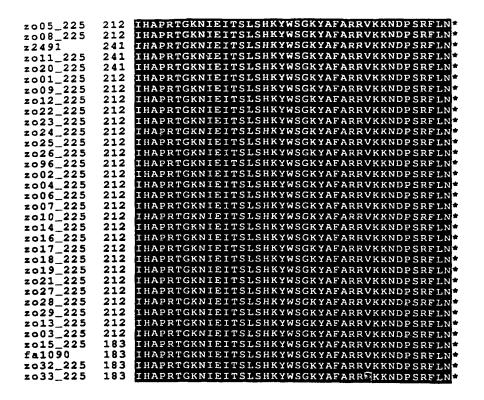


Fig. 19C

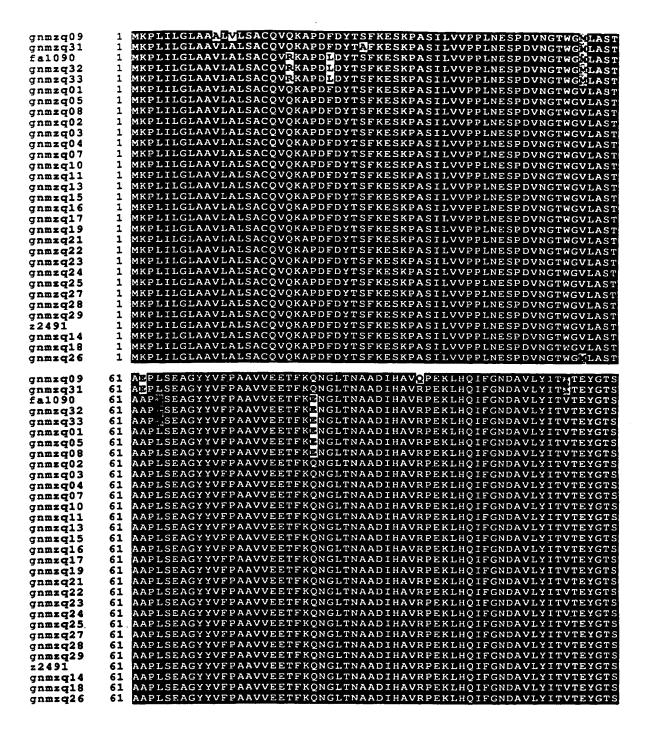


Fig. 20A

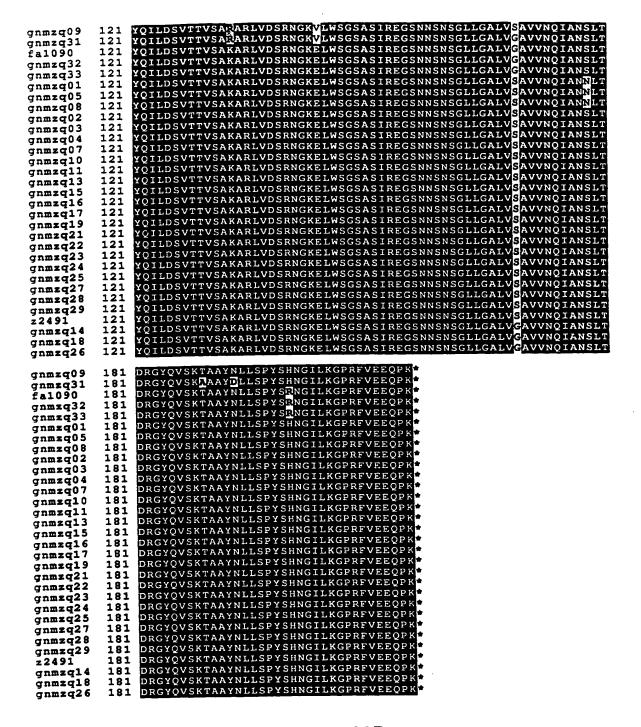
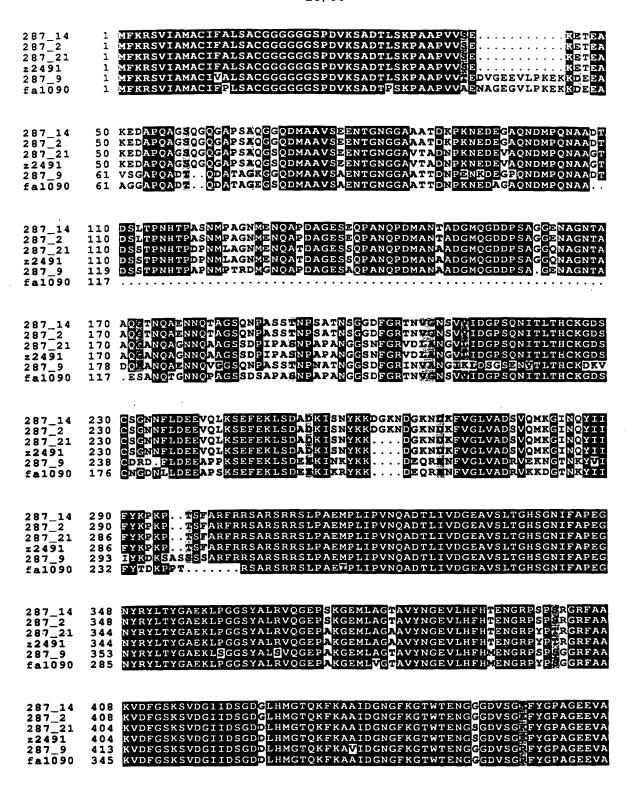


Fig. 20B

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**FIG. 21A** 

PCT/US99/09346

287_14	468	GKYSYRPTDAEKGGFGVFAGKKEQD*
287_2	468	GKYSYRPTDAEKGGFGVFAGKKEQD*
287_21	464	GKYSYRPTDAEKGGFGVFAGKKEQD*
z2491	464	GKYSYRPTDAEKGGFGVFAGKKEQD*
287_9	473	GKYSYRPTDAEKGGFGVFAGKKEQD*
fa1090	405	GKYSYRPTDAEKGGFGVFAGKK DRD*

FIG. 21B

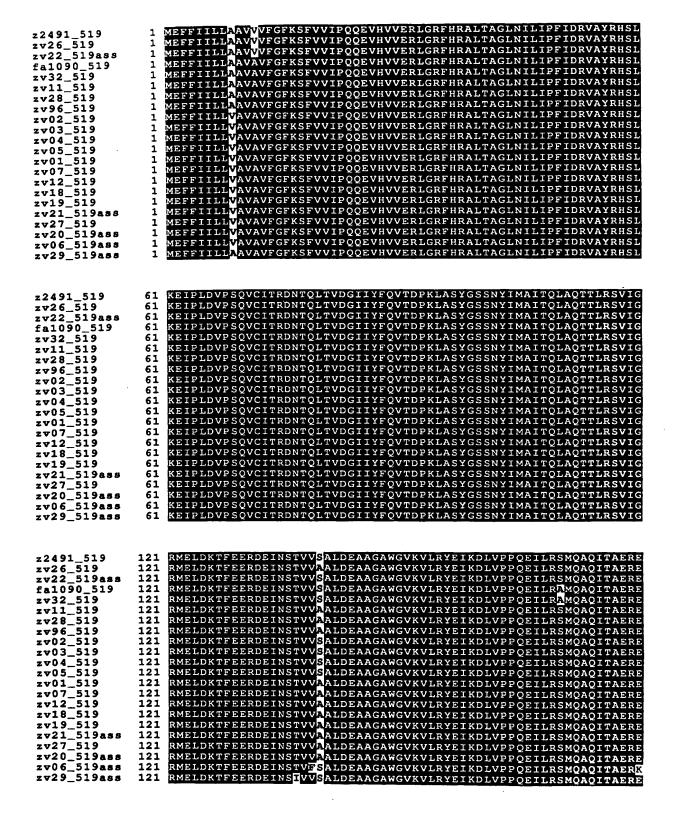


FIG. 22A

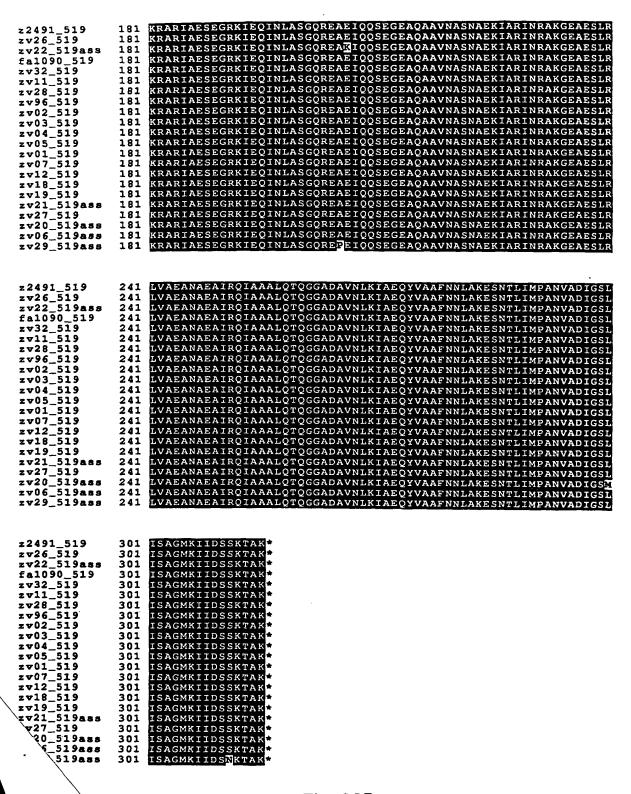


Fig. 22B

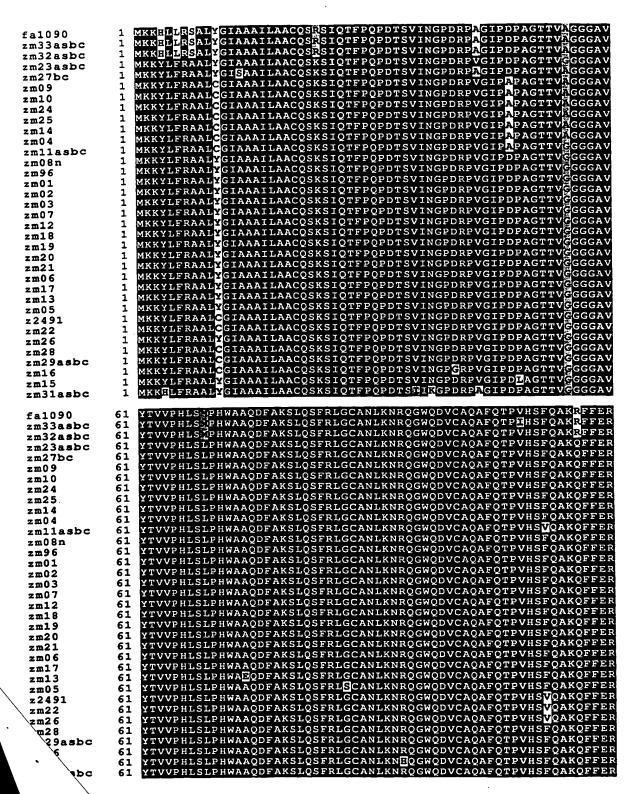


Fig. 23A

PCT/US99/09346 L

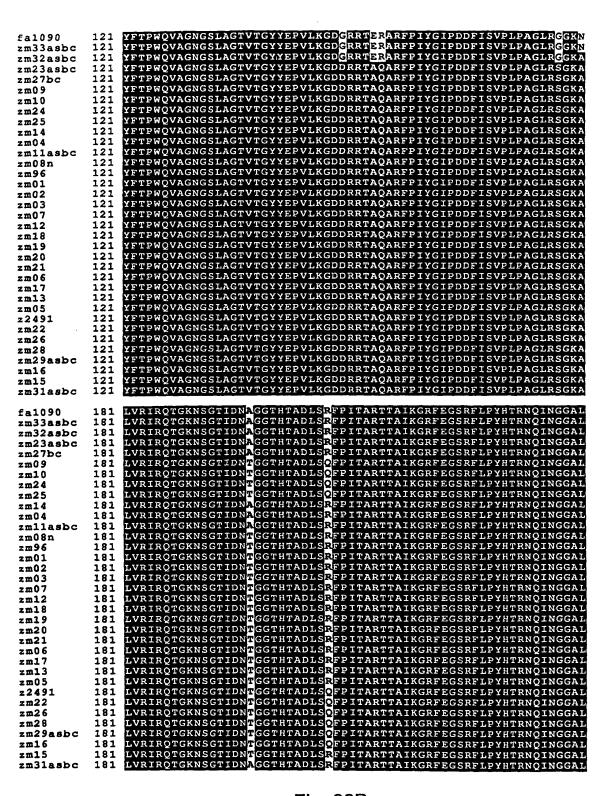


Fig. 23B

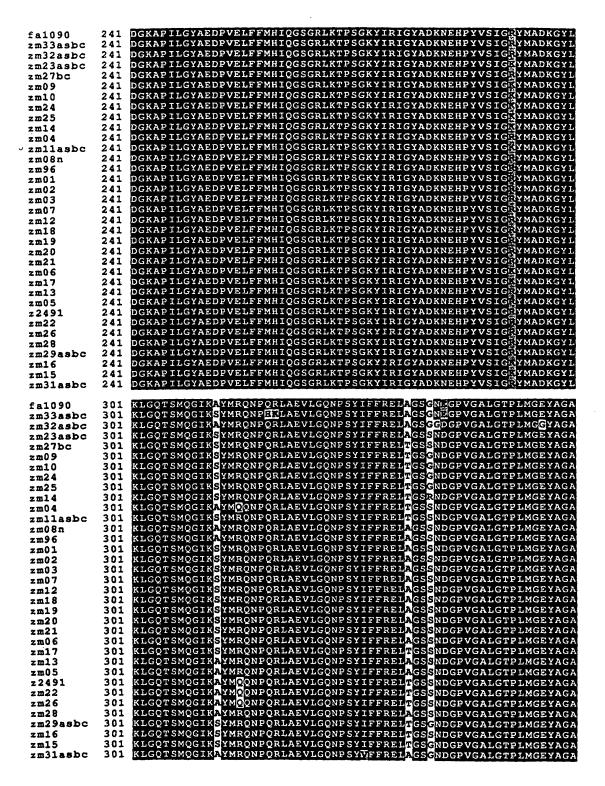


Fig. 23C

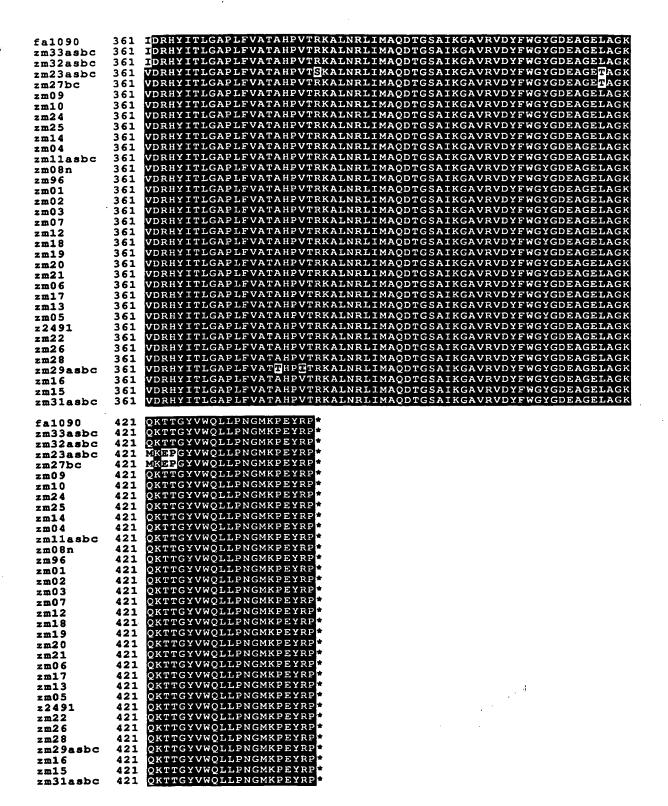


Fig. 23D